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Biochemical Characterization of Novel Tetrahydrofuranyl 1β-Methylcarbapenems: Stability to Hydrolysis by Renal Dehydropeptidases and Bacterial β-Lactamases, Binding to Penicillin Binding Proteins, and Permeability Properties

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The biochemical properties of tetrahydrofuranyl (THF) carbapenems, carbapenems with THF substituents, were evaluated with respect to enzyme stability, binding to penicillin-binding proteins (PBPs), and penetration into gram-negative organisms. THF carbapenems showed increased stability to hog renal dehydropeptidases (DHPs) compared to that of imipenem or meropenem and were more stable to human DHP than imipenem (<10% hydrolysis compared to that for imipenem). THF carbapenems were stable to hydrolysis by all serine β-lactamases tested. CL 191,121, a prototype THF carbapenem, was more stable to hydrolysis by carbapenem-hydrolyzing serine β-lactamases such as IMI-1 and Sme-1 than imipenem, with a relative k_{cat} value of <20% for imipenem. Similar to imipenem and meropenem, THF carbapenems were not stable to the metallo β-lactamases CcrA and L1. However, CL 191,121 bound to all *Staphylococcus aureus* PBPs at concentrations that were less than or equal to the MICs. The THF carbapenems bound to PBPs from *Escherichia coli* and *Pseudomonas aeruginosa*, with the highest affinities being for PBPs 2 and 4, as noted with imipenem. The affinities for PBPs 1a and 1b in *E. coli* were reduced for the THF carbapenems compared to that for imipenem, even though the MICs of the THF carbapenems for *E. coli* strains were lower than those of imipenem. The penetrability of the THF carbapenems into *Serratia marcescens* S6, which produces the Sme-1 carbapenem-hydrolyzing β-lactamase, was 2.4 to 7.8 times less than that of imipenem. Compounds CL 190,294 and CL 188,624 showed good penetrability, with permeability coefficient values comparable to those of the rapidly penetrating agents cephaloridine, imipenem, meropenem, and biapenem. Decreased penetration into wild-type *P. aeruginosa* was suggested by the high MICs of the THF carbapenems (MICs, 16 to 32 μg/ml), despite equivalent or better binding to *P. aeruginosa* PBPs than that of imipenem. However, the MICs of the THF carbapenems for wild-type *P. aeruginosa* compared to that for an OprD2 mutant generally varied no more than 2-fold, but those of imipenem and other carbapenems differed 16-fold. These data indicated that THF carbapenems do not appear to enter through protein OprD2. In conclusion, the THF carbapenems exhibited stability to hydrolysis by renal DHPs and serine β-lactamases, exhibited strong binding to essential PBPs from *E. coli* and *S. aureus*, and penetrated gram-negative enteric bacteria at rates comparable to those for meropenem and biapenem.

Carbapenems, such as imipenem, meropenem, and biapenem, have the broadest spectrum of antimicrobial activity among the β-lactams and exhibit fewer common resistance problems (2, 20, 23). This activity is due to the combined effects of good stability to hydrolysis by most β-lactamases, strong binding to essential penicillin-binding proteins (PBPs), and good penetrability into gram-negative organisms (8, 29, 30). The excellent activity of imipenem against *Pseudomonas aeruginosa* has also been attributed to entry into the organisms via a specific carbapenem uptake pathway involving the OprD protein channel (30). However, imipenem can easily be destroyed by mammalian dehydropeptidases (DHPs) (13, 19) and must be administered with the DHP inhibitor cilastatin (11). In addition, imipenem has a relatively short half-life in vivo and is not orally active. With a 1,β-methyl group attached to the β-lactam nucleus, meropenem and biapenem showed significantly improved stability to DHP hydrolysis (9, 35). However, none of these carbapenems are orally active.

Tetrahydrofuranyl (THF) carbapenems, such as CL 191,121, CL 188,624, and CL 190,294, are a new class of carbapenems with THF substituents (15–17). These carbapenems exhibit broad-spectrum antibacterial profiles that combines the good activities of meropenem and biapenem against gram-negative bacteria with the excellent activity of imipenem against gram-positive bacteria (15, 31). A series of peptidic THF carbapenem prodrugs such as CL 191,638 and CL 191,983 are based on the parent compound CL 191,121 and exhibit activity when they are administered orally (16, 32). They are especially attractive because both imipenem and meropenem are parenterally administered drugs. The present study outlines the biochemical properties of THF carbapenems for their stabilities to the hydrolytic activities of DHPs from the hog, mouse, rat, and human and to bacterial serine and metallo β-lactamases. The PBP affinities for THF carbapenems were compared with those for other carbapenems. The penetration properties of THF carbapenems into gram-negative bacteria have also been evaluated.

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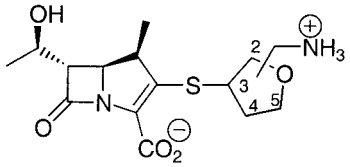


FIG. 1. Structures of the aminomethyl THF 1-β-methyl carbapenems. Stereochemistries were as follows (*R* and *S* define the configuration of the chiral carbon atom): CL 191,121, 3*R*, 2*R*; CL 188,624, 3*S*, 5*R* and 3*R*, 5*S*; CL 190,294, 3*R*, 5*R* and 3*S*, 5*S*.

MATERIALS AND METHODS

Antibiotics. Biapenem, piperacillin, THF carbapenems, and their derivatives were synthesized by the Chemistry Group of Wyeth-Ayerst Research. The structural characteristics of each THF carbapenem are indicated in Fig. 1 and Table 1 (also, see references 15 to 17). Imipenem was obtained from Merck (Rahway, N.J.), meropenem was obtained from Zeneca (Maccelesfield, England), cephaloridine was obtained from Sigma (St. Louis, Mo.), and cefotaxime was obtained from Hoechst-Roussel Pharmaceuticals Inc. (Frankfurt, Germany). Working solutions of antimicrobial agents were freshly prepared on each day of assay.

Microorganisms. *Serratia marcescens* S6, a carbapenem-hydrolyzing serine β-lactamase producer (33), was used as the test organism for the penetration assays. *P. aeruginosa* 27853 was used to derive the OprD2-deficient mutants. A standard *P. aeruginosa* OprD2-deficient isolate, isolate GC 1543, originated by Quinn et al. (24), was also used for comparison. *Staphylococcus aureus* 29213, *Escherichia coli* MC4100, and *P. aeruginosa* 27853 were used as test organisms for the PBP assays.

Enzymes. DHPs from fresh hog and mouse kidney and from rat intestine were extracted with butanol and were precipitated with ammonium sulfate in 10 mM HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-2 ethanesulfonic acid) buffer (pH 7.2) (7). The enzymes were solubilized from frozen precipitates on each day of assay. A human DHP gene was cloned from a human kidney cDNA gene bank and was expressed in human kidney 293 cells (25). DHP was partially purified as described previously (1).

Bacterial metallo β-lactamase CcrA from *Bacterioids fragilis* was purified to homogeneity from the CcrA cloned in *E. coli* DH5α (34). Metallo β-lactamase L1 from *Stenotrophomonas maltophilia* was purified by CM-C50 cation-exchange chromatography (4). The serine β-lactamases, P99 from *Enterobacter cloacae*, PC1 from *S. aureus*, TEM-2 from *E. coli*, TEM-26 from *Klebsiella pneumonia*, and Sme-1 from *S. marcescens*, were purified to homogeneity by previously described methods (35). The IMI-1 β-lactamase from *E. cloacae* was purified to homogeneity by column chromatography (26).

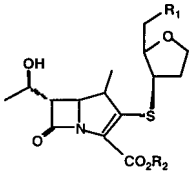
Enzyme stability and kinetic studies. Carbapenems were prepared at a concentration of 1.0 mg/ml in water and were assayed at a final concentration of 50 μg/ml. The relative hydrolysis rates for carbapenems were determined spectrophotometrically at UV wavelengths of 290 to 300 nm on the basis of the maximum change in absorbance in the difference spectrum following enzymatic hydrolysis by the CcrA enzyme. The hydrolysis of carbapenems by the DHPs and metallo β-lactamases was measured in 10 mM HEPES buffer (pH 7.2). Hydrolysis of carbapenems by serine β-lactamases was determined in 50 mM phosphate buffer (pH 7.0). Two different volumes of enzyme (10 to 50 μl) were used in a total volume of 1,000 μl, and rates were determined as nanomoles of substrate hydrolyzed per microliter of enzyme solution added. Imipenem and meropenem were included as reference compounds for each set of assays. Relative hydrolysis rates were calculated by normalizing the specific molar hydrolysis rates to those observed with imipenem on the same day. Kinetic parameters (K_m and K_{cat}) of the carbapenem-hydrolyzing enzymes were derived with the ENZPACK (Biosoft) program on the basis of two independent experiments with at least six concentrations of the substrate at a single enzyme concentration. The maximum hydrolytic activity was reported as a k_{cat} value (second^{-1}) for each substrate. The total protein concentration in each enzyme preparation was determined by a bicinchoninic acid assay (Pierce Chemical Co., Rockford, Ill.).

Assay for PBP binding. PBP binding was assessed by competition assays based on the method of Spratt (28). In standard assays, carbapenems were incubated with solubilized membranes for 10 min at 30°C. [^{14}C]benzylpenicillin was added to give a final concentration of 10 μg/ml. The membranes were incubated for another 10 min at 30°C, and the reaction was terminated with cold acetone. For mechanistic studies the timing and temperatures of the incubations were varied (3). PBPs were detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography. X-ray films were analyzed on a Shimadzu CS9000U densitometer, with the 50% inhibitory concentrations (IC_{50}s) determined graphically.

Penetration studies. The penetrabilities of the β-lactams into gram-negative bacteria were determined by the method introduced by Zimmermann and Rosselet (37) and modified by Nikaido et al. (22). *S. marcescens* S6, the Sme-1 carbapenem-hydrolyzing serine β-lactamase producer, was used as the test organism. Details of the permeability assay were described previously (35). The antibiotic diffusion rate (VE) was obtained with whole-cell suspensions. The hydrolysis rates were determined with sonicated cells and were used to derive K_m and V_{max} values. Permeability coefficients were calculated by the formula originated by Nikaido et al. (22).

Derivation and identification of *P. aeruginosa* OprD2 mutants. *P. aeruginosa* 27853 was cultured (10^8 CFU) on a tryptic soy agar plate containing 4, 8, and 16 μg of imipenem per ml. Plates containing 4 and 8 μg of imipenem per ml were incubated at 37°C for 24 h, and plates containing 16 μg of imipenem per ml were incubated for 48 h. Resistant selection frequency was calculated on the basis of the growth of colonies on the imipenem-containing plates. The imipenem resistance of the isolated colonies was confirmed by disc diffusion testing and MIC assays. Outer membrane proteins of the parent and selected resistant isolates, as

TABLE 1. Stabilities of THF carbapenems to hydrolysis by renal DHPs and metallo β-lactamases[a]



Carbapenem	R_1	R_2	Relative hydrolysis rate (%)[b]					
			DHP				β-Lactamase	
			Mouse kidney	Hog kidney	Human kidney	Rat intestine	CcrA	L1
Imipenem			100	100	100	100	100	100
Meropenem			210	24	5.3	55	530	210
Biapenem			48	5.4	1.7	ND[c]	25[d]	28[d]
CL 188,624	NH_2 *trans*, 3,5-	H	58	8.4	8.5	ND	760	170
CL 190,294	NH_2 *cis*, 3,5-	H	26	5.4	1.7	ND	490	160
CL 191,121	NH_2	H	30	10	4.4	102	85	80
CL 191,638	NH-L-Ala	H	140	14	7.6	180	370	110
CL 191,983	NH-L-Val	H	27	8.4	16	160	92	24
CL 191,986	NH-L-Ile	H	ND	ND	4.8	195	120	42

[a] The structure below represents the generic structure of THF carbapenems.
[b] Relative initial hydrolysis rate for carbapenems tested at 50 μg/ml. The rates were calculated by normalizing the specific molar hydrolysis rates to those observed for imipenem on the same day.
[c] ND, not determined.
[d] Data are from Yang et al. (35).

TABLE 2. Kinetic parameters of carbapenem-hydrolyzing β-lactamases for THF carbapenems[a]

Enzyme	Substrate	k_{cat} (s^{-1})	Relative k_{cat}	K_m (μM)	k_{cat}/K_m (mM^{-1} s^{-1})	Relative k_{cat}/K_m
CcrA	Imipenem	15	100	63	240	100
	Meropenem	8.4	56	38	220	92
	Biapenem	3.7	25	200	19	7.9
	CL 188,624	68	450	17	4,000	1,600
	CL 190,294	40	270	21	1,900	800
	CL 191,121	8.7	58	25	350	150
L1	Imipenem	2.0	100	26	76	100
	Meropenem	0.88	44	11	80	110
	Biapenem	0.55	28	19	29	38
	CL 188,624	1.5	77	22	69	91
	CL 190,294	0.9	47	10	91	120
	CL 191,121	1.8	91	24	76	100
Sme-1	Imipenem	0.33	100	66	5.0	100
	Meropenem	0.028	8.5	4.3	6.5	130
	Biapenem	0.034	10	5.4	6.3	130
	CL 188,624	0.026	7.9	11	2.4	48
	CL 190,294	0.021	6.4	14	1.5	30
	CL 191,121	0.061	19	43	1.4	28
IMI-1	Imipenem	100	100	170	530	100
	Meropenem	10	10	26	380	71
	Biapenem	9.4	9.4	32	290	55
	CL 188,624	9.7	9.3	17	570	110
	CL 190,294	8.3	8.0	23	370	70
	CL 191,121	7.1	6.8	24	710	130

[a] Data for meropenem and biapenem are from Yang et al. (35). Data for IMI-1 are from Rasmussen et al. (26).

well as those of the control OprD2 deficient strain, were extracted with 1% laurylsarcosine and identified by SDS-PAGE with 12% polyacrylamide gels (27).

Microbiological assays. MICs were determined by the broth microdilution method in Mueller-Hinton II broth as recommended by the National Committee for Clinical Laboratory Standards (21).

RESULTS

Stabilities of THF carbapenems to DHPs. The relative hydrolysis rates of DHP and metallo β-lactamases for the THF carbapenems, together with their structural characteristics, are summarized in Table 1. For hog DHPs, all the THF carbapenems were hydrolyzed at rates slower than those observed for imipenem and meropenem. The stabilities of carbapenems to hydrolysis by mouse and human DHPs indicated that all compounds tested were less stable to mouse DHPs than to hog DHPs, especially meropenem and CL 191,638. However, for human DHP, all THF carbapenems showed excellent stability compared to that of imipenem, with a rate <20% that for imipenem. The hydrolysis of the THF carbapenems CL 191,121, CL 191,638, and CL 191,983 by rat intestine enzymes proceeded at a rate comparable to or faster than that for hydrolysis of imipenem (Table 1).

Stabilities to carbapenem-hydrolyzing β-lactamases. Many metallo β-lactamases can hydrolyze all classes of β-lactams including carbapenems. The THF carbapenems, including the hydrophilic compounds with a hydrogen or amino group at the terminus of one or both of the side chains, were also not stable to the hydrolysis by the two metallo β-lactamases CcrA and L1 (Table 1).

The kinetic parameters for hydrolysis of selected THF carbapenems with carbapenem-hydrolyzing β-lactamases are summarized in Table 2. As initially indicated by the relative hydrolysis rate (Table 1) for the metallo β-lactamase CcrA, all THF carbapenems except CL 191,121 were less stable than imipenem on the basis of the k_{cat} value; CL 191,121 was almost twofold more stable than imipenem. For the metallo β-lactamase L1, all THF carbapenems were as stable as or slightly more stable than imipenem on the basis of the k_{cat} value. K_m values with the CcrA enzyme were lower for THF carbapenems than for imipenem, resulting in higher k_{cat}/K_m values. However, on the basis of k_{cat} values, THF carbapenems were much more stable than imipenem to hydrolysis by the carbapenem-hydrolyzing serine β-lactamases Sme-1 and IMI-1. The k_{cat} values of Sme-1 and IMI-1 for the THF carbapenems were 5 to 16 times slower than those for imipenem. Although lower K_m values were observed for THF carbapenems than for imipenem with IMI-1 and Sme-1 (Table 2), the catalytic efficiencies (k_{cat}/K_m values) were still lower for the THF carbapenems than for imipenem with the Sme-1 enzyme.

Stabilities to other serine β-lactamases. All THF carbapenems showed excellent stability to hydrolysis by serine β-lactamases in functional groups 1, 2a, 2b, and 2e (molecular classes C and A) (Table 3), with the relative hydrolysis rate for the THF carbapenems never exceeding 1% of that for the reference compound. The extended-spectrum β-lactamase TEM-26 hydrolyzed all the carbapenems slowly.

Affinities of THF carbapenems for PBPs. THF carbapenems bound to PBPs from both gram-positive and gram-negative organisms, including *P. aeruginosa*, with affinities comparable to those of other carbapenems (Table 4). The good antimicrobial activities of the THF carbapenems against *S. aureus* (31) were consistent with tight binding to PBP 1 (Table 4). Moreover, CL 191,121 bound well to all PBPs from *S. aureus*. None of the THF carbapenems bound well to PBP 2a from methicillin-resistant *S. aureus* BB270 (MICs, >16 μg/ml), with the IC_{50}s of all compounds being >100 μg/ml (data not shown). With *E. coli* all three THF carbapenems exhibited similar PBP profiles, with excellent binding to PBPs 2 and 4 (IC_{50}s, <0.1

TABLE 3. Stabilities of THF carbapenems to hydrolysis by serine β-lactamases

β-Lactam	Relative hydrolysis rate (%) by the following enzyme (functional group)[a]:			
	P99 (1)	PC1 (2a)	TEM-2 (2b)	TEM-26 (2be)
Cephaloridine[b]	100	ND[c]	ND	ND
Benzylpenicillin	ND	100	100	100
Imipenem	0.01	≤0.01	0.03	0.28
CL 188,624	0.12	≤0.01	0.02	0.27
CL 190,294	0.01	≤0.01	0.01	0.29
CL 191,121	0.29	≤0.01	0.16	0.60

[a] Relative hydrolysis rate was calculated on the basis of 100% hydrolysis of the reference β-lactam. Functional groups are those of Bush et al. (6).
[b] Cephaloridine was selected as the reference compound for the group 1 β-lactamase P99; benzylpenicillin was selected as reference compound for the group 2 β-lactamases.
[c] ND, not determined.

μg/ml). CL 191,121 had better affinity for PBPs 1a and 1b than CL 188,624 and CL 190,294, but it still bound to these two PBPs less effectively than imipenem or meropenem did. The last two THF carbapenems bound to PBP 3 better than any of the carbapenems tested other than meropenem. Despite the reduced antimicrobial activity against *P. aeruginosa*, the THF carbapenems exhibited strong binding to the PBPs in *P. aeruginosa*, having comparable or better IC_{50}s than those of imipenem and biapenem for PBPs 1b, 1c, 2, and 3 (Table 4).

When assayed at 30°C, poor binding to PBP in *S. aureus* was seen for CL 188,624 and CL 190,294 (IC_{50}s were estimated to be 5 and 20 μg/ml, respectively), whereas IC_{50}s were 0.5 and 0.15 μg/ml, respectively, at 0°C. However, the IC_{50} of CL 191,121 (0.06 μg/ml) was much lower at 30°C and was comparable to the IC_{50} determined at 0°C (0.12 μg/ml). Apparent affinities were improved 10- to 100-fold for CL 188,624 and CL 190,294 when assay temperatures were decreased to 0°C. As seen previously with selected monobactams (3), this differential in IC_{50}s as a function of temperature suggests that rapid deacylation of the first two isomers can occur at 30°C. Better binding to PBP 1 of *S. aureus* was observed at the higher assay temperature. This is consistent with direct kinetic measurements indicating a slow acylation rate for PBP 1 (half-life of 2.5 to 3.2 min at 30°C).

Penetration of THF carbapenems into *S. marcescens* S6. The permeability coefficients of representative β-lactams and THF carbapenems CL 188,624, CL 190,294, and CL 191,121 are summarized in Table 5. Cephaloridine and imipenem are compounds that penetrate gram-negative organisms fast, as demonstrated here and by other researchers (22, 36). Piperacillin and cefotaxime penetrated the organisms more slowly. The THF carbapenems, especially CL 190,294 and CL 188,624, showed good penetrability through the porin channels of *S. marcescens* S6, with permeability coefficients comparable to that of the rapidly penetrating compound cephaloridine. CL 191,121 penetrated more slowly than the other two THF carbapenems tested but still exhibited a rate approximately 30% of that for cephaloridine. The THF carbapenems CL 190,294 and CL 188,624 were transported into *S. marcescens* at rates similar to those for meropenem and biapenem.

Selection and identification of OprD2-deficient mutants. Imipenem-resistant mutants of *P. aeruginosa* 27853 were selected at a frequency of 10^{-7}. Resistance to imipenem with the selected mutants was confirmed by disc diffusion tests. Outer

TABLE 4. Binding of carbapenems to PBPs of *S. aureus*, *E. coli*, and *P. aeruginosa*

Organism	PBP	Concn (μg/ml) required to inhibit binding of [^{14}C]benzylpenicillin by 50% or MIC (μg/ml)[a]					
		Imipenem	Meropenem	Biapenem	CL 188,624	CL 190,294	CL 191,121
S. aureus 29213	1	0.02	0.03	0.01	0.01	0.03	0.006
	2	0.10	0.18	0.06	0.1	0.2	0.04
	3	0.18	0.80	0.05	5.0	20[b]	0.06
	4	0.06	0.08	0.06	0.1	0.15	0.02
	MIC[a]	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06
E. coli 25922	1a	0.07	0.2	1.0	3.0	6.0	0.5
	1b	0.10	0.2	1.0	3.0	6.0	0.5
	2	0.05	0.02	0.003	0.03	0.03	0.05
	3	2.0	0.4	2.5	0.5	0.4	8.0
	4	0.07	0.02	0.01	0.05	0.06	<0.1
	5/6	3.9	17	90	50	40	>10
	MIC	0.12	<0.06	<0.06	<0.06	<0.06	<0.06
P. aeruginosa 27853	1b	0.37	0.5	0.06	0.33	0.07	0.04
	1c	0.29	0.5	0.40	0.40	0.30	0.20
	2	0.43	0.03	0.18	0.04	0.10	0.05
	3	0.42	0.04	0.30	0.40	0.04	0.2
	4	10	0.03	0.03	0.6	0.07	<0.1
	5/6	20	>100	90	>10	>10	>10
	MIC	2.0	0.5	0.50	32	32	16
P. aeruginosa 27853a	MIC	32	8	8	32	32	16
P. aeruginosa GC1543[d]	MIC	16	16	16	64	64	16

[a] Note that MICs were determined by the standard broth microdilution methodology (21).
[b] Estimate.
[c] Isogenic OprD2-deficient mutant from *P. aeruginosa* 27853 selected with imipenem.
[d] OprD2-deficient isolate (isolate B from Quinn et al. [24]).

TABLE 5. Penetration of novel THF carbapenems into *S. marcescens* S6[a]

Antibiotic	MIC (μg/ml)	Permeability coefficient (nm/s)	Relative permeability coefficient
Cephaloridine	>256	78	100
Imipenem	>256	180	230
Meropenem	64	83	110
Biapenem	64	69	89
Piperacillin	16	≤4[b]	≤5
Cefotaxime	0.25	≤5[b]	≤7
CL 188,624	>64	60	77
CL 190,294	>64	74	95
CL 191,121	64	23	30

[a] A clinical isolate from the London Hospital that produces a chromosomally mediated β-lactamase and the Sme-1 carbapenem-hydrolyzing enzyme (33).
[b] Estimated on the basis of the maximum entry rate detectable.

membrane extraction with laurylsarcosine and by protein identification by SDS-gel electrophoresis indicated that these imipenem-selected mutants shared the same outer membrane profile as the control OprD2-deficient strain: a loss of the OprD2 protein compared to the outer membrane protein profile of the parent strain (Fig. 2). The MICs of imipenem, meropenem, biapenem, and THF carbapenems for the OprD-deficient mutants in comparison with those for the wild-type parent are summarized in Table 4. For imipenem, meropenem, and biapenem, the MICs for the resistant mutants were elevated 8- to 32-fold compared to that for the parent strain, *P. aeruginosa* 27853. The MICs of the THF carbapenems were never more than twofold higher for the OprD2 mutants compared to those for the wild-type strain.

DISCUSSION

Early carbapenems such as imipenem were not stable to hydrolysis by the zinc-containing enzymes such as mammalian renal DHPs and metallo-β-lactamases. One of the most important features that a novel carbapenem needs to compete in the commercial marketplace is improved stability to DHPs. As with meropenem, the addition of a 1-β-methyl group to the β-lactam nucleus of THF carbapenems such as CL 191,121 and CL 191,983 increased the stability to mammalian DHPs.

β-Lactamase production is one of the major mechanisms of bacterial resistance to β-lactams. Although carbapenems are generally quite stable to hydrolysis by most common β-lactamases, they are generally not stable to the class B metallo β-lactamases. Therefore, it is still essential that the stabilities of novel carbapenems to a battery of β-lactamases from various sources be evaluated. The evaluation of THF carbapenems for hydrolysis by the metallo-β-lactamases CcrA and L1 indicated that hydrophilic compounds containing a free carboxylic group were less stable to hydrolysis by the metallo β-lactamases. Carbapenem-hydrolyzing serine β-lactamases such as IMI-1 and Sme-1 were capable of hydrolyzing imipenem and meropenem. However, THF carbapenems such as CL 191,121 were more stable than imipenem to hydrolysis by IMI-1 and Sme-1. In addition, THF carbapenems such as CL 191,121 were stable to hydrolysis by the other serine β-lactamases tested.

The three compounds selected for more extensive evaluation demonstrated that the good penetrability of the THF carbapenems, especially CL 190,294 and CL 188,624, through the porin channels of *S. marcescens* S6, combined with their high degrees of affinity to PBPs 2 and 4 of gram-negative organisms (5), contributed to their good activities against gram-negative bacteria.

The modest antipseudomonal activities of the THF compounds were also studied. Although the penetrabilities of the THF carbapenems into members of the family *Enterobacteriaceae* were comparable to those of other carbapenems, penetration into *P. aeruginosa* appeared to be diminished. The levels of binding to essential PBPs in *P. aeruginosa* were comparable for biapenem and the THF carbapenems (5), but the MICs of the THF carbapenems were higher. This study demonstrated that the loss of the OprD2 protein channel had little effect upon the antipseudomonal activities of the THF carbapenems but markedly affected the activities of imipenem and biapenem (eightfold or greater increases in MICs). The differences in the MICs of the THF carbapenems for parent strain *P. aeruginosa* 27853 were not more than twofold compared to those for its OprD2-deficient mutants. These results indicate that THF carbapenems do not appear to use the specific imipenem-penetrating channel OprD2 as their major route for entry into *P. aeruginosa*. This behavior is similar to that reported for a series of carbapenems bearing a basic group at either position 1 or position 6 (e.g., BMY 45047); imipenem has a single basic group at position 2 (10). Decreased activity in *P. aeruginosa* appeared to be due to poor uptake through protein OprD2.

Imipenem resistance in *P. aeruginosa* reflects a complex interplay between inducibility and stability to group 1 (class C) β-lactamases, uptake through porins, especially OprD2 (18), and efflux potential (12, 14). The THF carbapenem CL 191,121 behaved as a weaker inducer than imipenem, with at least a fivefold lower induction ratio than that for imipenem at a concentration of one-half the MIC (data not shown). Unfortunately, the stabilities of imipenem and CL 191,121 to hydrolysis by crude enzyme extracts from *P. aeruginosa* ATCC 27852 and the OprD2-deficient mutants were not measurable due to the relative stabilities of both compounds and the low enzyme concentrations in the extracts. However, with a homogeneously purified AmpC β-lactamase from *E. cloacae*, CL 191,121 was almost fourfold more stable than imipenem at 100 μM (data not shown). These data suggest that, in addition to not using the OprD2 channel, the better stability of the THF carbapenems to hydrolysis by an AmpC β-lactamase may contribute to the minimal effect on the THF carbapenem MICs for imipenem-resistant mutants. These findings correspond to Liver-

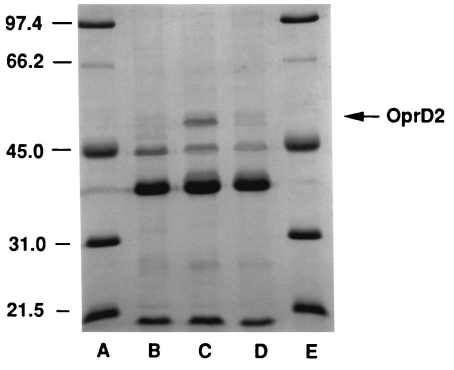


FIG. 2. Outer membrane protein profiles of *P. aeruginosa* strains. Lanes A and E, molecular mass markers (Bio-Rad, Richmond, Calif.); lane B, isolate B of an OprD2-deficient strain (24); lane C, *P. aeruginosa* ATCC 27853; lane D, OprD2-deficient mutant from *P. aeruginosa* 27853 selected with imipenem. Numbers on the left are in kilodaltons.

more's (18) comments that "the activity of a carbapenem more β-lactamase stable than imipenem should be less affected by the porin loss." The efflux potentials for THF carbapenems have not been evaluated, whereas those for the carbapenem ER-35786 and for various β-lactams have been evaluated by Köhler et al. (12) and Li et al. (14), respectively.

The good stability to hydrolysis by DHP and β-lactamases, efficient binding to the target proteins, and favorable penetrability coupled with their in vitro and in vivo antimicrobial activities support the further evaluation of THF carbapenems. These THF carbapenems are under investigation as orally active compounds.

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